REMARKS

This amendment is submitted in response to the Notice to Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures mailed June 12, 2002. In the Notice, the Office stated that the subject application clearly fails to comply with the requirements of 37 C.F.R. 1.821 - 1.825, and that Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing", an initial paper or compact disc copy of the "Sequence Listing", and an amendment directing its entry into the application.

In response to the Notice to Comply, submitted herewith are initial computer readable form (CRF) and initial paper copies of the "Sequence Listing", as well as instructions submitted herein that direct entry of the two copies into the application. In addition, the content of the Sequence Listing information recorded in computer readable form is identical to the written Sequence Listing.

Applicants respectfully submit that all of the sequences contained in the Sequence Listing were disclosed in the Specification and/or Drawings as originally submitted on December 18, 2001. The Specification and Drawings have been amended herein to clearly identify each sequence by a SEQ ID NO identifier. Therefore, Applicants respectfully submit that the initial CRF and paper copies of the Sequence Listing submitted herewith do not contain any

new matter. In addition, Applicant respectfully submits that no substantive changes have been made in the application, and the amendments to the Specification and Drawings do not introduce any new matter. Applicant respectfully requests that such amendments be entered into the record.

Should the Examiner have any questions regarding this Response, or the remarks contained herein, Applicant's agent would welcome the opportunity to discuss such matters with the Examiner.

Respectfully submitted,

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MARKED-UP VERSION OF PARAGRAPHS OF THE SPECIFICATION

SHOWING THE CHANGES MADE THERETO

[0162] Of the 44 peptide sequences listed in Table 4, it is noteworthy that overlaps across other HLA-B15 molecules are evident within our data collection. The B*1510 tapasin$_{354-362}$ ligand HHSDGSVSL **(SEQ ID NO:51)**, as well as both THTQPGVQL **(SEQ ID NO:54)** from septin 2 homolog$_{70-78}$ and SHANSAVVL **(SEQ ID NO:55)** from β-adaptin$_{249-257}$, have also been sequenced from B*1509 extracts (Barber et al. 1997), and the B*1501/B*1508/B*1512 ubiquitin-protein ligase$_{83-91}$-derived ligand ILGPPGSVY **(SEQ ID NO:41)** was characterized from endogenously bound B*1502 peptides (Barber et al. 1997). The eIF3-p66$_{61-69}$ nonamer SQFGGGSQY **(SEQ ID NO:29)** (Falk et al. 1995; Barber et al. 1996) was found here within B*1501, B*1503, B*1508, and B*1512 extracts. The decamer YMIDPSGVSY **(SEQ ID NO:42)**, which is homologous to proteasome subunit C8$_{150-159}$, was also previously described as a ligand for B*1502 (Barber et al. 1997), B*1508 (Barber et al. 1997), and B*4601 (Barber et al. 1996); it was found here presented by B*1501, B*1508, and B*1512. Some of the specific overlapping ligands identified in this study therefore overlap in antigen presentation with the HLA-B15 allotypes characterized by others.

[0184] A further example provided here of how C-proximal auxiliary anchors might positively impact endogenous ligand binding is that eight of the

peptides overlapping both the B*1508 and B*1501 antigen binding grooves bear Thr at C^{-1}, C^{-2}, or C^{-3}, and in four cases the peptides that bind B*1508/B*1501 or B*1508/B*1501/B*1503 are heptamers with Thr occupying P7, their C-terminal positions (FIG. 26). The prominent role of Thr as a C-terminal/proximal auxiliary anchor is dramatically illustrated by the B*1508/B*1501/B*1503 overlapping heptamer CPLSCFT **(SEQ ID NO:60)**, where Thr provides a C-terminal anchor for this ligand not evident in the pooled motifs of the three allotypes.

[0185] Distilling the data from the overlapping ligands among B*1501, B*1503, and B*1508 suggests a model for endogenous ligand binding whereby peptides are first anchored or held in the class I binding groove by their C termini. In order for a given peptide to remain stably fastened in the groove for successful trimer assembly and subsequent export from the cell, it is observed that following rigid anchoring at the C terminus as described, a ligand must be subsequently tethered into the class I antigen binding cleft at a more variably defined N-proximal position. Such is the case for peptide ligand NQZHGSAEY **(SEQ ID NO:138)**, a nonamer that overlaps across B*1508, B*1501, and B*1503 (FIG. 24). According to this model (FIG. 27), a C-terminal Tyr securely anchors NQZHGSAEY **(SEQ ID NO:138)** into all three B15 allotypes, while a Gln at P2 anchors the peptide into B*1501 and B*1503 and a Gln/Lys (most likely a Lys based upon both motif assignments and

fractional Edman sequencing data) at P3 provides additional anchoring for B*1501 and serves as the sole N-proximal anchor for B*1508. This model appears clearly applicable to at least 75% of the ligands presented in FIG. 26; for those peptides to which it does not evidently apply, the possible anchoring modes remain open to further speculation at the level of individual ligands.

[0186] For example, the B*1501/B*1503 overlap AQFASGAGZ **(SEQ ID NO:135)** (FIG. 26) may instead be additively stabilized through the N-proximal anchors indicated at P2 and P3 as well as at the N-terminal position, since Ala demonstrated significant P1 occupancy among both B*1501 and B*1503 ligands, as previously shown (FIGS. 21 and 22, A). Additionally, the four heptameric overlaps that were observed across B*1508/B*1501/B*1503, which terminate in Thr, could lie within the peptide binding groove such that they are anchored N-terminally/proximally and their C termini interact with the C-proximal regions of the groove, which have demonstrated preferences for Thr; these ligands might therefore fail to extend into the F-pocket. As compared with C-terminal sequences, both length and N-proximal specificity characteristics of ligands generally play secondary roles in the natural binding of B15 peptide epitopes.

[0189] Though functionally divergent according to its pooled motif (FIG. 16) and the majority of peptides that it binds, B*1510 is capable of accommodating ligands with the properties favored by the B*1501, B*1503,

and B*1508 binding grooves. Data both from individual ligands (Table D) and fractional Edman sequencing (Table 3) indicate that Tyr can occupy the C-terminal position, and specific examples in Table 4, including the spleen mitotic checkpoint $BUB3_{53-60}$ octamer YQHTGAVL **(SEQ ID NO:32)** and the splicing factor U2AF large $chain_{179-187}$ nonamer TQAPGNPVL **(SEQ ID NO:37)**, attest to B-pocket flexibility. It is intriguing that among the peptides bound by B*1510 is the $tapasin_{354-362}$ nonamer HHSDGSVSL **(SEQ ID NO:51)**; the peptide appears to occupy ligand extracts in a high copy number, as qualitatively based upon relative mass spectrometric ion intensities. Given this, as well as considering potential models of loading complex interactions suggested by others (Neisig et al. 1996; Elliott 1997), it can be extrapolated that a portion of tapasin, analogous to class II-associated invariant chain-derived peptides (Riberdy et al. 1992; Sette et al. 1992), extends into and blocks a region of the empty class I binding groove until it is displaced by an optimally-fitting ligand and/or secondary chaperone; this could also account for the differences in overall P2 flexibility observed between B*1510 peptides and those of the other three allotypes. Participating in ligand selection by this mechanism would describe a distinct peptide editing role for tapasin and could clarify the inability to detect overlaps between B*1510 and either B*1501, B*1503, or B*1508.

[0195] To illustrate the potential consequences of applying the modified search parameters described, the EBV structural antigen gp85, which has

recently been implicated using a murine model as a favorable target against which protective CTLs might be generated (Khanna et al. 1999), was examined in the context of B*1501 to identify: (i) nonameric epitopes with motif-prescribed P2 and P9 occupancies; (ii) length variant epitopes with motif-prescribed P2 and P9 occupancies; and (iii) nonameric epitopes with flexible P2 occupancy (Table 16). Since only these three categories of ligands were designated, the inquiry was not exhaustive. However, the information extracted showed that, of the 98 possible epitopes identified, only the 22% within the first column would be placed under further experimental consideration if pooled motifs alone were applied in the search. This is not to imply that the data in this category is invalid but that it might be considerably incomplete for later applications. For example, if either of the $AMTSKFLMGTY_{172\text{-}182}$ **(SEQ ID NO:205)**(varying by length) or the $SAPLEKQLF_{123\text{-}131}$ **(SEQ ID NO:246)**(varying by P2 occupancy) peptides was demonstrated to elicit a more effective antigen-specific CTL response than any of the nonamers bearing standard motif P2/P9 assignments, this knowledge is pivotal to subsequent vaccine design; even if the two designated peptides evoked responses only equivalent to some of the nonamers, their non-motif length and/or sequences discrepancies could prove superior in conferring the ability to overlap multiple allotypes in addition to B*1501. This is advantageous since a vaccine consisting of a single or limited number of peptide specificities

could theoretically be effective for protecting populations differing in HLA type (Loftus et al. 1995; Gulukota et al. 1996; Sidney et al. 1996c).

[0351] The three following oligonucleotide primers, designated A, B, and C, were purchased from commercially available sources (i.e. operon):

Primer A, a 5' primer:
5'__GGGCCTCGAGGGACTCAGAATCTCCCCAGACGCCGAG __3'
(SEQ ID NO:634)

Primer B, a 3' primer:
5'__CCGCAAGCTTCCATCTCAGGGTGAGGGGCT__3'
(SEQ ID NO:635)

Primer C, a 3' primer:
5'__CCGCGAATTCTTATTCGTGCCATTCGATTTTCTG__3'
(SEQ ID NO:636)

[0352] Primers A and B were used in PCR #1 and the template cDNA was a recombinant sHLA-B*0702 truncated gene with a 6-histidine tail in pcDNA3.1(-), a mammalian expression vector. PCR #1 was designed to incorporate 5' *Xho* I (CTC GAG) and 3' *Hin*d III (AAG CTT) cut sites and to amplify the truncated B*0702 heavy chain lacking a 3' stop codon. All PCR reactions for this project were performed with a proofreading taq polymerase (PFU Polymerase, Promega) and were subjected to 25 cycles of 95°C for 1 minute (strand separation), 59°C for 1 minute (primer annealing), and 72°C for 2 minutes (DNA synthesis), followed by a final extension time of 7 minutes at 72°C. The product of PCR #1 was purified using a QIAquick PCR purification kit (QIAGEN) and was subsequently digested for 2 hr at 37°C with *Xho* I and *Hin*d III. Concurrently, a bacterial expression vector (C-Terminal Biotin AviTag Vector,

Avidity; catalog number pAC-6) (FIG. 41) encoding the biotinylation substrate peptide sequence GLNDIFEAQKIEWHE **(residues 3-17 of SEQ ID NO:631)** was digested with the same restriction enzymes under the same conditions. The digest products were gel purified using a Freeze N Squeeze kit (BioRad) and ligated together for 2.5 hours at room temperature. Competent cells of *E. coli* strain JM1⌀9 were transformed with the ligated DNA, plated on LB/ampicillin agar, and incubated for 16 hours at 37°C. Using colony PCR, colonies were screened for insert of the gene into the vector.

[0354] Cells of the human cell line T2 were cultured in RPMI 1640 media + 20% fetal calf serum, 1% penicillin/streptomycin, and 0.25% phenol red. A total of 1.7 X 10^7 T2 cells were transfected with 30 µg of B*0702t-bsp/pcDNA3.1(-) DNA by electroporation using the Gene Pulser (BioRad) at 0.25 V and 960 µFD. Transfected cells were selected in a medium containing 40% RPMI 1640, 40% conditioned media, 20% fetal calf serum, 2% penicillin/streptomycin, 0.2% phenol red, and 1.5 mg/mL G418 neomycin (Cellgro). Surviving cells were pulsed with the synthetic HIV GAG peptide NH_2-S-P-R-T-L-N-A-W-V-COOH **(SEQ ID NO:637)** at 20 ug/mL and then incubated for 24 hours at 37°C. Transfectants were then screened by ELISA using W6/32 (8 µg/mL), which is directed against the entire HLA heavy chain/light chain (β_2m)/peptide complex, as the primary (capture) antibody and anti-β_2m conjugated to HRP (diluted 1:1000) as the secondary (conjugate) antibody

(Dako).

[0359] The construct successfully created using the AviTag bsp vector contained a 7-residue sequence (W-K-L-P-A-G-G) **(SEQ ID NO:638)** between the truncated B7 heavy chain at its C-terminus and the bsp. Despite the fact that the peptide binding groove is at the N-terminus of the heavy chain, it was undetermined at the time of transfection whether this 7-residue linker would in any way affect MHC protein folding or peptide binding capability. Because class I molecules must be properly folded and loaded with peptide before being directed to the cell surface, only the specific MHC/peptide complexes should be in the supernatant. Additionally, the W6/32 monoclonal capture antibody of the ELISA positively identified the presence of MHC-bsp/peptide complexes secreted into the supernatant by producing transfectants (FIG. 44). Thus, the 7 residue linker did not alter protein folding or peptide loading.